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Light Microscopy and Phylogenetic Analyses of *Chlamydomonas* Species (Volvocales, Chlorophyceae). II. Molecular Phylogeny, Secondary Structure of ITS-2, Cell Morphology, and Nomenclature of *Microglena opisthopyren*, and *M. media*, comb. nov.

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Two species of *Chlamydomonas* Ehrenb. in the clade *Monadinia, Chlamydomonas pseudomacrostigma* L. Ş. Péterfi ex H. Ettl and *C. media* G. A. Klebs, were recently reported from Japan. However, several species in the clade were transferred to the genus *Microglena* Ehrenb. by different authors, and therefore the taxonomic treatment of these two species should be revisited. Here, their identifications were re-examined based on 18S *r*RNA gene phylogeny, secondary structure comparisons of ITS-2 rDNA, and vegetative cell morphology. Based on these comparisons, *C. pseudomacrostigma* and *C. media* were treated as a later synonym of *Microglena opisthopyren* (Skuja) Demchenko, Mikhailyuk & Pröschold and an earlier synonym of *M. skujae* Demchenko, Mikhailyuk & Pröschold, respectively. A new combination, *Microglena media* (G.A. Klebs) Nakada, comb. nov., is proposed for the latter.

Keywords: *Chlamydomonas*, ITS-2, *Microglena*, molecular phylogeny, phycology, synonymy

Nakada *et al*. (2012) reported two species of *Chlamydomonas* Ehrenb., *C. pseudomacrostigma* L. Ş. Péterfi ex H. Ettl based on TkS0811B3 (=NIES-2744) and *C. media* G. A. Klebs based on HcCl-5-3 (=NIES-2743), from Japan, and examined their phylogenetic position using 18S *r*RNA genes. Subsequently, they were phylogenetically affiliated to the clade *Monadinia* in the order Volvocales. However, Demchenko *et al*. (2012) reclassified several species belonging to the clade as the genus *Microglena* Ehrenb., and proposed a new classification system based on light microscopy, 18S *r*RNA gene phylogeny, and the secondary structure of ITS-1 and -2. They also gave a DNA Barcode of *Microglena* species using ITS-2 DNA sequences. Two species reported by Nakada *et al*. (2012) clearly belong to the *Microglena*, and one strain of *C. pseudomacrostigma*, SAG 8.87, was already identified as *M. opisthopyren* (Skuja) Demchenko, Mikhailyuk & Pröschold by Demchenko *et al*. (2012). Here, the phylogenetic positions of *C. pseudomacrostigma* and *C. media* among the genus *Microglena* were therefore re-examined using 18S *r*RNA genes, and their species delineations were examined based on the secondary structure of ITS-2 and brief observations in addition to those of Demchenko *et al*. (2012) and Nakada *et al*. (2012). The phylogenetic position and ITS-2 secondary structure of an unidentified strain, *Chlamydomonas* sp. SAG 10.87, were also examined for reference. Based on the results of phylogenetic and structural re-analyses, the correct names for the examined strains are discussed.

Materials and Methods

Examined strains, DNA sequencing and phylogenetic analyses were performed as described in Nakada *et al.* (2012), with the following exceptions: 1) ITS-2 DNA of TkS0811B3 (=NIES-2744; AB872921), HcCl-5-3 (=NIES-2743; AB872922) and SAG 10.87 (AB872923) were amplified and sequenced using the primers reported in Coleman *et al.* (1994); 2) 18S *r*RNA gene sequences of *Microglena* for phylogenetic analyses were revised referring to Demchenko *et al.* (2012); and 3) Bayesian Markov chain Monte Carlo iterations were performed for 2,000,000 generations.

The secondary structure models of newly determined ITS-2 were constructed using the program mfold (Mathews *et al.* 1999, Zuker 2003; http://mfold.rna.albany.edu/?q=mfold/RNA-Folding-Form) and manually refined referring to the models of Demchenko *et al.* (2012).

Cultivation and light microscopy were as described in Nakada *et al.* (2012). Sexual reproduction of HcCl-5-3 and SAG 16.90 was also examined at 12°C (following Klebs 1896) and 20°C. Dense cultures in 30 mL AF-6 medium were condensed by centrifugation, washed once in 15 mL AF-6−N medium [AF-6 medium lacking nitrogen compounds (sodium nitrate and ammonium nitrate)] then incubated in 15 mL AF-6−N medium for 1 day. One-day-old cultures of the two strains were further incubated separately or as mixtures for 7 days and then observed. To confirm the formation or absence of resistant cysts, the 7-day-old cultures were centrifuged and dried on sterilized filter paper for 5 days in 60 × 15 mm petri dishes. Five mL AF-6 medium was then added to the petri dishes prior to incubation for 7 days. The presence or absence of germinated vegetative cells was confirmed under a binocular microscope (SL-60N; Vixen, Saitama, Japan) or by inoculation in 10 mL AF-6 medium in screw cap tubes (18 × 150 mm).

Results

Molecular phylogeny of 18S r*RNA*

In the Bayesian phylogenetic tree of *Microglena* species (Fig. 1), the strain TkS0811B3 (=NIES-2744) identified as *Chlamydomonas pseudomacrostigma* was most closely related to strains of *M. opisthopyren* [SAG 8.87, SAG 31.86 and CCAP 11/46 (=SAG 54.90)] with strong statistical support [1.00 posterior probability (PP) and 88–91% bootstrap proportions (BPs)]. The aligned region of 18S *r*RNA was identical among SAG 8.87, SAG 31.86 and CCAP 11/46 (=SAG 54.90), but different from TkS0811B3 (=NIES-2744) in three base pairs. The strain HcCl-5-3 (=NIES-2743) identified as *C. media* was most closely related to the strain *M. skujae* Demchenko, Mikhailyuk & Pröschold SAG 16.90 (1.00 PP and 100% BPs). The examined region of 18S rRNA was different between HcCl-5-3 (=NIES-2743) and SAG 16.90 in one base pair. The phylogenetic position of "*Chlamydomonas* sp." SAG 10.87 could not be resolved by Bayesian analysis alone. The 18S *r*RNA gene sequence was most similar to those of *M. globulifera* (Korshikov) Demchenko, Mikhailyuk & Pröschold and *M. indica* (M.O.P. Iyengar) Demchenko, Mikhailyuk & Pröschold, but they were different from SAG 10.87 in 10 base pairs.

Secondary structure of ITS-2

The secondary structure models of ITS-2 DNA of "*Chlamydomonas pseudomacrostigma*" TkS0811B3 (=NIES-2744) were compared with those of "*C. pseudomacrostigma*" SAG 8.87 (FR854386) and SAG 54.90 (FR854387), and that of "*C. media*" HcCl-5-3 (=NIES-2743) was compared with *Microglena skujae* SAG 16.90 (FR854389; Fig. 2). One compensatory base change (CBC) and three hemi-compensatory base changes (hemi-CBCs), but no base-pair insertions/deletions (indels) were found between TkS0811B3 (=NIES-2744) and SAG 8.87 (and SAG 54.90). Among the CBC/hemi-CBCs, only one hemi-CBC in the helix III was observed in the conserved DNA Barcode region indicated by

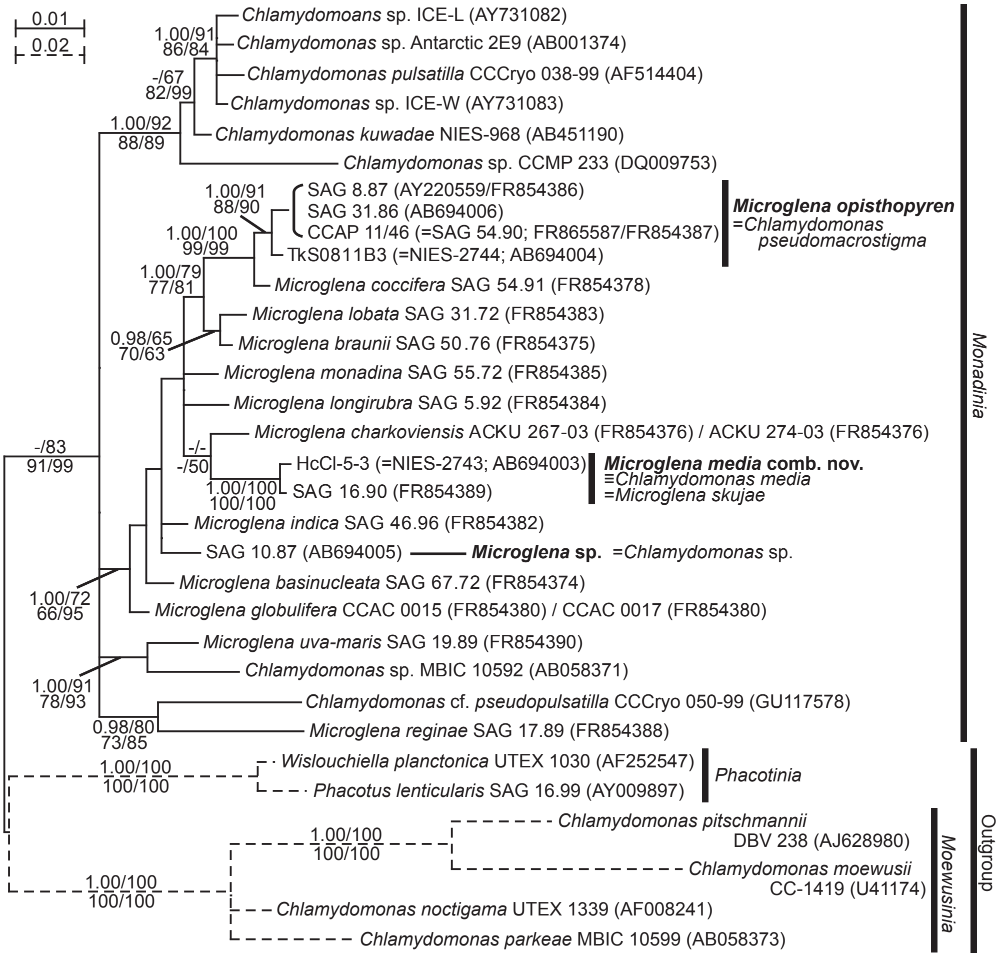
0.01
0.02
Chlamydomoans sp. ICE-L (AY731082)
Chlamydomonas sp. Antarctic 2E9 (AB001374)
Chlamydomonas pulsatilla CCCryo 038-99 (AF514404)
Chlamydomonas sp. ICE-W (AY731083)
Chlamydomonas kuwadae NIES-968 (AB451190)
Chlamydomonas sp. CCMP 233 (DQ009753)
1.00/91 86/84
-/67 82/99
1.00/92 88/89
SAG 8.87 (AY220559/FR854386)
SAG 31.86 (AB694006)
CCAP 11/46 (=SAG 54.90; FR865587/FR854387)
TkS0811B3 (=NIES-2744; AB694004)
Microglena opisthopyren
=Chlamydomonas pseudomacrostigma
1.00/91 88/90
1.00/100 99/99
Microglena coccifera SAG 54.91 (FR854378)
1.00/79 77/81
Microglena lobata SAG 31.72 (FR854383)
Microglena braunii SAG 50.76 (FR854375)
0.98/65 70/63
Microglena monadina SAG 55.72 (FR854385)
Microglena longirubra SAG 5.92 (FR854384)
Microglena charkoviensis ACKU 267-03 (FR854376) / ACKU 274-03 (FR854376)
-/- -/50
HcCl-5-3 (=NIES-2743; AB694003)
SAG 16.90 (FR854389)
1.00/100 100/100
Microglena media comb. nov.
≡Chlamydomonas media
=Microglena skujae
Microglena indica SAG 46.96 (FR854382)
SAG 10.87 (AB694005)
Microglena sp. =Chlamydomonas sp.
Microglena basinucleata SAG 67.72 (FR854374)
Microglena globulifera CCAC 0015 (FR854380) / CCAC 0017 (FR854380)
1.00/72 66/95
-/83 91/99
Microglena uva-maris SAG 19.89 (FR854390)
Chlamydomonas sp. MBIC 10592 (AB058371)
1.00/91 78/93
Chlamydomonas cf. pseudopulsatilla CCCryo 050-99 (GU117578)
Microglena reginae SAG 17.89 (FR854388)
0.98/80 73/85
Monadinia
Wislouchiella planctonica UTEX 1030 (AF252547)
Phacotus lenticularis SAG 16.99 (AY009897)
1.00/100 100/100
Phacotinia
Chlamydomonas pitschmannii DBV 238 (AJ628980)
Chlamydomonas moewusii CC-1419 (U41174)
1.00/100 100/100
Chlamydomonas noctigama UTEX 1339 (AF008241)
Chlamydomonas parkeae MBIC 10599 (AB058373)
1.00/100 100/100
Moewusinia
Outgroup

FIG. 1. Bayesian phylogenetic tree of the clade *Monadinia* (Volvocales) based on 18S *r*RNA gene sequences, showing the phylogenetic positions of *Microglena opisthopyren*, *M. media*, and *M.* sp. SAG 10.87 (shown in bold). Sequences of the clades *Phacotinia* and *Moewusinia* were used as the outgroup. The corresponding posterior probabilities (≥0.90; top left) and the bootstrap proportions (≥50%) from maximum likelihood (top right), maximum parsimony (bottom left), and neighbor-joining (bottom right) analyses are shown by the branches. Branch lengths and the scale bar represent the expected number of nucleotide substitutions per site. Accession numbers of the International Nucleotide Sequence Databases for each gene are indicated in parenthesis.

Demchenko *et al.* (2012). Five hemi-CBCs were found between HcCl-5-3 (=NIES-2743) and SAG 16.90, but no CBC and base-pair indels were found. Among the hemi-CBCs, one hemi-CBC in the helix III was observed in the conserved DNA Barcode region. Three unique base pairs among *Microglena* species in the conserved DNA Barcode region were shared between HcCl-5-3 (=NIES-2743) and SAG 16.90 (Fig. 2). An ITS-2 structure model of SAG 10.87 was also constructed. It contained three unique base pairs among *Microglena* species in the conserved DNA Barcode region (Fig. 2).

Morphological comparison and sexual reproduction of HcCl-5-3 and SAG 16.90

Vegetative cell morphology of the strains HcCl-5-3 and SAG 16.90 were compared under the same culture conditions. The cells of SAG 16.90 were slightly larger (11–24 μm in length

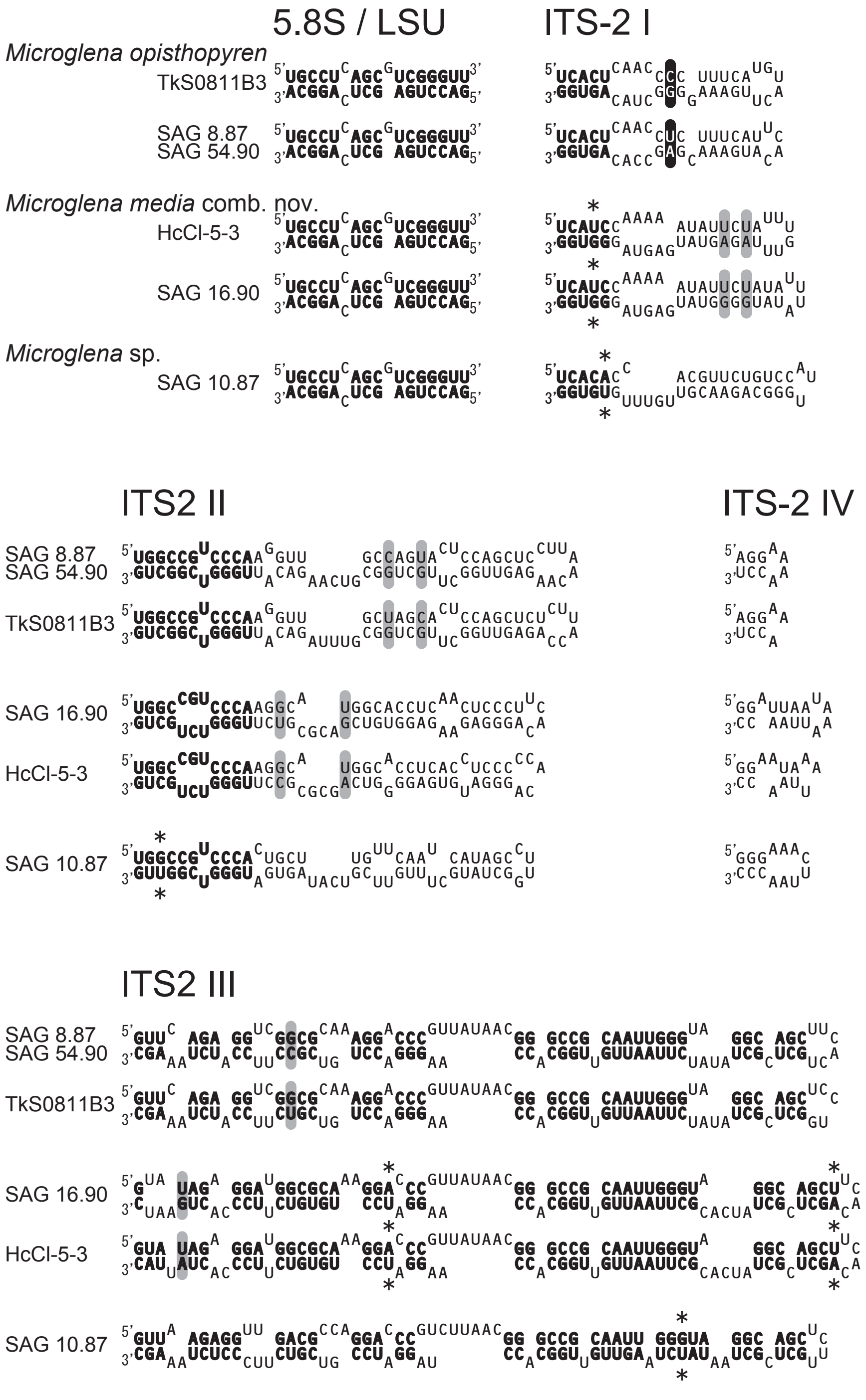
5.8S / LSU
ITS-2 I
Microglena opisthopyren
TkS0811B3
5' UGCCU C AGC G UCGGGUU 3'
3' ACGGA C UCG AGUCCAG 5'
5' UCACU CAAC CCC UUUCA UG U
3' GGUGA CAUC GGG G AAAGU UC A
SAG 8.87
SAG 54.90
5' UGCCU C AGC G UCGGGUU 3'
3' ACGGA C UCG AGUCCAG 5'
5' UCACU CAAC CUC UUUCAU U C
3' GGUGA CACC GAG C AAAGUA C A
Microglena media comb. nov.
HcCl-5-3
5' UGCCU C AGC G UCGGGUU 3'
3' ACGGA C UCG AGUCCAG 5'
*
5' UCAUC C AAAA AUAUUCUA UU U
3' GGUGG G AUGAG UAUGAGAU UU G
SAG 16.90
5' UGCCU C AGC G UCGGGUU 3'
3' ACGGA C UCG AGUCCAG 5'
*
5' UCAUC C AAAA AUAUUCUAUA U U
3' GGUGG G AUGAG UAUGGGGUAU A U
*
Microglena sp.
SAG 10.87
5' UGCCU C AGC G UCGGGUU 3'
3' ACGGA C UCG AGUCCAG 5'
*
5' UCACAC C ACGUUCUGUCC A U
3' GGUGUG UUUGU UGCAAGACGGG U
*
ITS2 II
ITS-2 IV
SAG 8.87
SAG 54.90
5' UGGCCG U CCCA A G GUU GCCAGUA CU CCAGCUC CUU A
3' GUCGGC U GGGU U A CAG AACUG CGGUCGU UC GGUUGAG AAC A
5' AGG A A
3' UCC A A
TkS0811B3
5' UGGCCG U CCCA A G GUU GCUAGCA CU CCAGCUCU CU U
3' GUCGGC U GGGU U A CAG AUUUG CGGUCGU UC GGUUGAGA CC A
5' AGG A A
3' UCC A
SAG 16.90
5' UGGC CGU CCCA AGGC A UGGCACCUC AA CUCCCU U C
3' GUCG UCU GGGU UCUG CGCA GCUGUGGAG AA GAGGGA C A
5' GG A UUAA U A
3' CC AAUU A A
HcCl-5-3
5' UGGC CGU CCCA AGGC A UGGC A CCUCAC C UCCC CC A
3' GUCG UCU GGGU UCCG CGCG ACUG G GGAGUG U AGGG AC
5' GG AA UA A A
3' CC A AU U
SAG 10.87
*
5' UGGCCG U CCCA C UGCU UG UU CAA U CAUAGC C U
3' GUUGGC U GGGU A GUGA UACU GC UU GUU UC GUAUCG G U
*
5' GGG AAA C
3' CCC AAU U
ITS2 III
SAG 8.87
SAG 54.90
5' GUU C AGA GG UC GGCG CAA AGG A CCC GUUAUAAC GG GCCG CAAUUGGG UA GGC AGC UU C
3' CGA AA UCU A CC UU CCGC UG UCC A GGG AA CC A CGGU U GUUAAUUC UAUA UCG C UCG UC A
TkS0811B3
5' GUU C AGA GG UC GGCG CAA AGG A CCC GUUAUAAC GG GCCG CAAUUGGG UA GGC AGC UC C
3' CGA AA UCU A CC UU CUGC UG UCC A GGG AA CC A CGGU U GUUAAUUC UAUA UCG C UCG GU
SAG 16.90
*
*
5' G UA UAG A GGA U GGCGCA AA GGA C CC GUUAUAAC GG GCCG CAAUUGGGU A GGC AGCU U C
3' C UAA GUC AC CCU U CUGUGU CCU A GG AA CC A CGGU U GUUAAUUCG CACUA UCG C UCGA C A
HcCl-5-3
*
*
5' GUA UAG A GGA U GGCGCA AA GGA C CC GUUAUAAC GG GCCG CAAUUGGGU A GGC AGCU U C
3' CAU U AUC AC CCU U CUGUGU CCU A GG AA CC A CGGU U GUUAAUUCG CACUA UCG C UCGA C A
*
*
SAG 10.87
*
5' GUU A AGAGG UU GACG CCA GGA C CC GUCUUAAC GG GCCG CAAUU GGGUA GGC AGC U C
3' CGA AA UCUCC CUU CUGC UG CCU A GG AU CC A CGGU U GUUGA A UCUAU AA UCG C UCG U U
*

FIG. 2. Structures of ITS-2, including the stem of 5.8S and LSU rDNA, of *Microglena opisthopyren*, *M. media* and *M.* sp. SAG 10.87. Structures were predicted using mfold (Mathews *et al.* 1999, Zuker 2003) and manually refined by referring to the models of Demchenko *et al.* (2012). The strain *M. opisthopyren* TkS0811B3 (=NIES-2744) is compared with SAG 8.87 and SAG 54.90, and the strain *M. media* HcCl-5-3 (=NIES-2743) with SAG 16.90. Compensatory base changes and hemi-compensatory base changes are marked in black and gray, respectively. Conserved DNA Barcode regions defined by Demchenko *et al.* (2012) are indicated in bold. In the conserved DNA Barcode regions, base pairs unique among *Microglena* species are indicated by asterisks.

and 8–18 µm in width) than HcCl-5-3 (13–20 µm and 7–13 µm, respectively; Nakada *et al*. 2012), and the stigmata of SAG 16.90 were longer than those of HcCl-5-3. Other diagnostic characters examined (Demchenko *et al*. 2012, Nakada *et al*. 2012) were not significantly different.

Sexual reproduction was not observed in HcCl-5-3 or SAG 16.90 at both 12°C (following Klebs 1896) and 20°C, regardless of independent and mixed cultivation. No sexual reproduction was observed at either 12°C or 20°C. No cells survived after 5 days' desiccation, indicating an absence of resistant cyst formation.

Discussion

The phylogenetic tree supported the conclusion of Nakada *et al*. (2012) that the strains TkS0811B3 (=NIES-2744), SAG 8.87, SAG 31.86, and CCAP 11/46 (=SAG 54.90) are the same species. One ITS-2 CBC and three hemi-CBCs were observed among these strains. Nakada *et al*. (2010) and Hayama *et al*. (2010) found three CBCs and/or base-pair indels of ITS-2 between sister species, and at most, one CBC of ITS-2 within a species. Although the mutation level of ITS-2 might differ between groups of volvocalean algae, the observed ITS-2 differences between TkS0811B3 and the strains SAG 8.87 and SAG 54.90 did not conflict with their conspecific status. These strains, TkS0811B3 (=NIES-2744), SAG 8.87 and SAG 31.86, were morphologically indistinguishable from each other (Nakada *et al*. 2012), while the morphology of *Microglena coccifera* (Gorozh.) Demchenko, Mikhailyuk & Pröschold was clearly different from that of *M. opisthopyren* (Demchenko *et al*. 2012). TkS0811B3, on which the epitype of *C. pseudomacrostigma* is based (but see *Nomenclature and synonymy*), is therefore the same species as *M. opisthopyren*. Accordingly, because the epithet "*opisthopyren*" has priority, *C. pseudomacrostigma* should be treated as a later synonym of *M. opisthopyren*.

Nakada *et al*. (2012) cited *Chlamydomonas monadina* F. Stein var. *charkoviensis* Korshikov as a synonym of *C. pseudomacrostigma* (now *Microglena opisthopyren*); however, Demchenko *et al*. (2012) considered it an independent species, *M. charkoviensis* (Korshikov) Demchenko, Mikhailyuk & Pröschold. Although the type of *M. charkoviensis* is problematic (the citation in the protologue, Korshikov 1938, was likely erroneous; see Nakada *et al*. 2012), identification of the strain *M. charkoviensis* ACKU 267-03 seems appropriate, and as a result, it should be treated as an independent species from *M. opisthopyren* (=*C. pseudomacrostigma*).

The strain HcCl-5-3 (=NIES-2743) was found to be closely related to the strain SAG 16.90, which was assigned to *Microglena skujae*. Although no CBC or base-pair indel was found between these strains, indicating their conspecificity, their published morphology was slightly different. The vegetative cells of HcCl-5-3 (=NIES-2743) were narrower (7–13 µm; Nakada *et al*. 2012) than those of SAG 16.90 (14–20 µm; Demchenko *et al*. 2012); however, the culture conditions in those studies were slightly different. Nakada *et al*. (2012) cultivated HcCl-5-3 in AF-6 medium (Kato 1982, modified as in Andersen *et al*. 2005) at 20°C, 130–200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, while Demchenko *et al*. (2012) cultivated SAG 16.90 in Bold's Basal Medium with Vitamins and Triple Concentrated N (=3N-BBM+V; Schlösser 1997) at 20°C, 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Under the same culture conditions, the ranges in cell size overlapped [13–20 × 7–13 µm (HcCl-5-3) and 11–24 × 8–18 µm (SAG 16.90)], and only a slight difference in stigma size was observed. These differences are trivial, and accordingly, one cannot clearly distinguish the strains based on these characters alone. Here, HcCl-5-3 and SAG 16.90 are therefore treated as the same species. Because the epithet "*media*" has priority over the epithet "*skujae*" (and "*nova*"), *C. media* was transferred to the genus making a new combination *Microglena media* (G.A. Klebs) Nakada for the correct name of this species.

Klebs (1896) reported isogamous sexual reproduction in *Microglena media*; however, Demchenko *et al*. (2012) observed advanced anisogamy in all four *Microglena* species whose strains showed sexual reproduction. This apparently contradicts identification of the strains HcCl-5-3 and SAG 16.90 as *Microglena media*. However, sexual reproduction among *Microglena* was observed in only four species among the 13 species recognized by Demchenko *et al*. (2012), and it is therefore premature to assume that isogamous sexual reproduction is absent in *Microglena* species. Sexual reproduction was not observed in HcCl-5-3 or SAG 16.90. It is therefore possible that they are heterothalic (and representing a single mating type), or sexually sterile due to years of cultivation.

Nomenclature and synonymy

In the latest *International Code of Nomenclature for Algae, Fungi, and Plants*, the *Melbourne Code*, the term “illustration” acceptable as a nomenclatural type under Art. 8.1 was newly defined as “a work of art or a photograph depicting a feature or features of an organism” (Art. 8.1, footnote; McNeill *et al*. 2012). Literally interpreted, a series of drawings (each drawing sometimes given a unique figure number) depicting several cells of a new species derived from a single culture or natural sample, might not be considered “an” illustration. However, this interpretation contradicts the established custom of microalgal research (*e.g.* Gerloff 1940, Skuja 1956, Ettl 1965, 1976), and it clearly violates the stability of many microalgal names. Thus, the usage of a series of drawings or photographs as an illustration (as a type) is retained in this study, following the Preamble 13 of the *Melbourne Code*, which dictates “where the consequence of rules are doubtful, established custom is followed” (McNeill *et al.* 2012).

Microglena opisthopyren (Skuja) Demchenko, Mikhailyuk & Pröschold in Eur. J. Phycol. 47: 286. 2012. ≡ *Chlamydomonas opisthopyren* Skuja in Nova Acta Regiae Soc. Sci. Upsal., ser. 4. 16(3): 130. 1956. — *Typus*. Skuja (1956), pl. 18, fig. 26 (lectotype, designated by Demchenko *et al*. 2012). Cryopreserved strain SAG 8.87 (epitype supporting the lectotype, designated by Demchenko *et al*. 2012).

= ***C****hlamydomonas macroplastida* J.W.G. Lund f. *macrostigma* Bourr. in Hydrobiologia. 3: 256. 1951. ≡ *Chlamydomonas pseudomacrostigma* L.Ş. Péterfi ex H. Ettl in Nova Hedwigia Beih. 49: 358. 1976. — *Typus*. Bourrelly (1951), figs. 33–34 (holotype). Nakada *et al*. (2012), fig. 1 (epitype, designated by Nakada *et al*. 2012). [–*Chlamydomonas pseudomacrostigma* L.Ş. Péterfi in Nova Hedwigia 16: 223. 1968. nom. nud. (lacking a direct reference to the page in the original description by Bourrelly 1951)]

Distribution. France (Bourrelly 1951), Romania (Péterfi 1968), Ukraine (Korshikov 1938, Massjuk *et al*. 2011), United Kingdom (SAG 8.87, SAG 31.86, SAG 54.90 and CCAP 11/46; SAG, http://www.epsag.uni-goettingen.de/; CCAP, http://www.ccap.ac.uk/) and Japan (Nakada *et al*. 2012).

Strains. TkS0811B3 (=NIES-2744), SAG 8.87, SAG 31.86, SAG 54.90 and CCAP 11/46 (=SAG 54.90).

Remarks. If the strain TkS0811B3 (NIES-2744) is separated from *M. opisthopyren*, the specific epithet “*pseudomacrostigma*” will be used under the genus *Microglena*.

Microglena media (G. A. Klebs) Nakada, **comb. nov**. ≡ *Chlamydomonas media* G. A. Klebs in Fortpfl. Alg. Pilz.: 424. 1896; Nakada et al. in Acta Phytotax. Geobot. 62: 73, 2012. — *Typus*. Klebs (1896), fig. 12 (holotype). Nakada *et al*. (2012), fig. 3B (epitype, designated by Nakada *et al*. 2012).

= *Chlamydomonas media* G. A. Klebs var. *eustigma* Gerloff in Arch. Protistenkunde 94: 362. 1940. — *Typus*. Gerloff (1940), figs. 30–31 (holotype).

= *Chlamydomonas nova* Skuja in Nova Acta Regiae Soc. Sci. Upsal., ser. 4. 16(3): 128. 1956. ≡ *Microglena skujae* Demchenko, Mikhailyuk & Pröschold in Eur. J. Phycol. 47: 285. 2012. nom. illeg. — *Typus*. Skuja (1956), fig. 19 (lectotype, designated by Demchenko *et al*. 2012). Cryopreserved strain SAG 16.90 (epitype supporting the lectotype, designated by Demchenko *et al*. 2012). [–“*Chlamydomonas nova* Skuja” in Sörensen, Acta Limnol. 1: 49, 50, 68, 1948. nom. nud. (lacking description and type)]

Distribution. Common in Europe, including the former USSR (Ettl 1976, 1983, Massjuk *et al*. 2011), and Japan (Nakada *et al*. 2012).

Strains. HcCl-5-3 (=NIES-2743) and SAG

16.90.

Remarks. If the strain SAG 16.90 is separated from *M. media*, the specific epithet "*nova*" will be used under the genus *Microglena*. Demchenko *et al*. (2012) proposed *M. skujae* Demchenko *et al*. for *Chlamydomonas nova*, but the former name is superfluous.

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